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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	19.05.2005
Applicant's or agent's file reference: 15685PCT00	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK2004/000001	07.01.2004	07.01.2003

Applicant
SYMPHOGEN A/S, et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Rauf, A Tel. +49 89 2399-7548

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 15685PCT00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/DK2004/000001	International filing date *(day/month/year)* 07.01.2004	Priority date *(day/month/year)* 07.01.2003
International Patent Classification (IPC) or national classification and IPC C12N15/10		
Applicant SYMPHOGEN A/S, et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.08.2004	19.05.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Mandl, B Telephone No. +49 89 2399-8434

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000001

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-65 as originally filed

Sequence listings part of the description, Pages

1-2 as originally filed

Claims, Numbers

1-40 received on 18.12.2004 with letter of 18.12.2004

Drawings, Sheets

1/12-12/12 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 41-47
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-40
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-40
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 02/055718 (GENETASTIX CORP); 18 July 2002
D2: WO 02/44361 (APPLIED MOLECULAR EVOLUTION INC.); 6 June 2002

The subject-matter of claims 1-40 is novel over the available prior art (**Article 33(2) PCT**) and appears to involve an inventive step (**Article 33(3) PCT**) for the following reasons:

i. The present application relates to the manufacturing of recombinant polyclonal proteins by generating a collection of cells wherein each cell has site-specifically integrated into its genome a nucleic acid which encodes one distinct member of the polyclonal proteins. The integrated nucleic acid derives from a library of vectors, each encoding a single member of the polyclonal proteins and having one or more recombinase recognition sites which correspond to recombinase recognition sequences present in the cells.

ii. Polyclonal proteins, in particular monoclonal antibodies, are important therapeutics. They are either prepared from blood of human donors or by mixing monoclonal antibodies. The present application provides a manufacturing system which is not dependent on human blood and which allows the production in a few bioreactors as a single preparation.

iii. Even though, the methods used in the application were known in the prior art (see for example D1 and D2), it is considered inventive, because it does not appear obvious to combine the methods in order to arrive at the manufacturing system of the present application.

CLAIMS

1. A method for generating a collection of cells suitable as a recombinant polyclonal manufacturing cell line, said method comprising:

a) providing a library of vectors comprising a population of variant nucleic acid sequences, wherein each of said vectors comprises 1) one single copy of a distinct nucleic acid sequence encoding a distinct member of a polyclonal protein comprising distinct members that bind a particular antigen and 2) one or more recombinase recognition sequences;

b) introducing said library of vectors into a host cell line, wherein the genome of each individual cell of said host cell line comprises recombinase recognition sequences, matching those of the vector, at a single specific site in its genome;

c) ensuring the presence in said cells of one or more recombinases so that the variant nucleic acid sequences of step (a) are integrated site-specifically in the cells of the host cell line, where said one or more recombinases is/are either i) expressed by said cells into which said nucleic acid sequence is introduced; ii) operatively encoded by the vectors of step a; iii) provided through expression from a second vector; or iv) provided to the cell as a protein; and

d) selecting cells comprising an integrated copy from said library of variant nucleic acid sequences.

2. The method according to claim 1, wherein the polyclonal protein is not naturally associated with said collection of cells.

3. The method according to claim 1 or 2, wherein said polyclonal protein is a polyclonal antibody or antibody fragment.

4. The method according to claim 1 or 2, wherein said polyclonal protein is a polyclonal T cell receptor or T cell receptor fragment.

5. The method according to any one of the preceding claims, wherein said library of vectors is introduced into said host cell line by bulk transfection of a collection of said host cells with said library of vectors.

6. The method according to any one of claims 1-4, wherein said library of vectors is introduced into said host cell line by semi-bulk transfection of aliquots of said host cells with fractions comprising 5 to 50 individual vectors of said library of vectors, and said cells are pooled to form a collection of cells suitable as a recombinant polyclonal manufacturing cell line prior or subsequent to the selection of step (d).

7. The method according to any one of claims 1-4, wherein said library of vectors for site-specific integration is introduced into said host cell line by transfecting said host cells separately with individual members of said library of vectors, and said cells are pooled to form a collection of cells suitable as a recombinant polyclonal manufacturing cell line prior or subsequent to the selection of step (d).

8. The method according to any one of the preceding claims, wherein the population of variant nucleic acids in step (a) are isolated or identified by the aid of a screening procedure that enables identification and/or isolation of nucleic acids that encode protein which bind said particular antigen.

9. The method according to claim 8, wherein the screening procedure includes a biopanning step and/or an immunodetection assay.

10. The method according to claim 8 or 9, wherein said screening procedure is selected from the group consisting of phage display, ribosome display, DNA display, RNA-peptide display, covalent display, bacterial surface display, yeast surface display, eukaryotic virus display, ELISA and ELISPOT.

11. The method according to any one of the preceding claims, wherein said library of variant nucleic acid sequences comprises at least 3 variant nucleic acid sequences.

12. The method according to any one of the preceding claims, wherein individual members of said library of variant nucleic acid sequences are integrated in a single predefined genomic locus of individual cells in said collection of cells, said locus being capable of mediating high-level expression of each member of said recombinant polyclonal protein.

13. The method according to any one of the preceding claims, wherein each distinct nucleic acid sequence comprises a pair of gene segments that encode a member of a polyclonal protein comprised of two different polypeptide chains.

14. The method according to claim 13, wherein said pair of gene segments comprise an antibody heavy chain variable region encoding sequence and an antibody light chain variable region encoding sequence.

15. The method according to claim 13, wherein said pair of gene segments comprise a T cell receptor alpha chain variable region encoding sequence and a T cell receptor beta chain variable region encoding sequence.

16. The method according to claim 13, wherein said pair of gene segments comprise a T cell receptor gamma chain variable region encoding sequence and a T cell receptor delta chain variable region encoding sequence.

17. The method according to any one of the preceding claims, wherein said library of variant nucleic acid sequences comprises a naturally occurring diversity located within the variant nucleic acid sequences.

18. The method according to claim 17, wherein the naturally occurring diversity is located in CDR regions present in said variant nucleic acid sequences.

19. The method according to any one of the preceding claims, wherein said collection of cells is derived from a mammalian cell line or cell type.

20. The method according to claim 19, wherein said mammalian cell line is selected from the group consisting of Chinese hamster ovary (CHO) cells, COS cells, BHK cells, YB2/0, NIH 3T3, myeloma cells, fibroblasts, HeLa, HEK 293, PER.C6, and cell lines derived thereof.

21. A method for the manufacture of a polyclonal protein, wherein said polyclonal protein comprises distinct members that bind a particular antigen, said method comprising:

a) providing a collection of cells comprising a library of variant nucleic acid sequences, where each of said nucleic acid sequences encode a distinct member of said polyclonal protein and where each of said nucleic acid sequences are integrated at the same, single site of the genome of each individual cell in said collection of cells;

b) culturing said collection of cells under conditions facilitating expression of said polyclonal protein; and

c) recovering said expressed polyclonal protein from the cell culture cells or cell culture supernatant.

22. The method according to claims 21, wherein the collection of cells in step (a) is generated according to the method of any one of claims 1-20.

23. The method according to claim 21 or 22, wherein the polyclonal protein is not naturally associated with said collection of cells.

24. The method according to any one of claims 21-23, wherein the library of variant nucleic acids in step (a) are isolated or identified in an earlier step by the aid of a screening procedure that enables identification and/or isolation of nucleic acids that encode protein which bind said particular antigen.

25. The method according to claim 24, wherein the screening procedure includes a biopanning step and/or an immunodetection assay.

26. The method according to claim 24 or 25, wherein said screening procedure is selected from the group consisting of phage display, ribosome display, DNA display, RNA-peptide display, covalent display, bacterial surface display, yeast surface display, eukaryotic virus display, ELISA, and ELISPOT.

27. The method according to any one of claims 21-26, wherein said polyclonal protein is a polyclonal antibody or antibody fragment.

28. The method according to any one of claims 21-26, wherein said polyclonal protein is a polyclonal T cell receptor or T cell receptor fragment.

29. The method according to any one of claims 21-28, wherein the relative expression levels of the variant nucleic acid sequences are monitored.

30. The method according to claim 29, wherein said expression levels are monitored at mRNA level and/or protein level.

31. The method according to claim 29 or 30, wherein the culturing in step (b) is terminated at the latest when the relative expression levels are outside a predetermined range.

32. A recombinant polyclonal manufacturing cell line comprising a collection of cells transfected with a library of variant nucleic acid sequences, wherein each cell in the collection is transfected with and capable of expressing one member of the library, which encodes a distinct member of a polyclonal protein that binds a particular antigen and which is located at the same single site in the genome of individual cells in said collection, wherein said nucleic acid sequence is not naturally associated with said cell in the collection.

33. The recombinant polyclonal manufacturing cell line according to claim 32, wherein said library of variant nucleic acid sequences encodes a polyclonal antibody or antibody fragment having a naturally occurring diversity among the individual members of said polyclonal antibody or antibody fragments.

34. The recombinant polyclonal manufacturing cell line according to claim 32, wherein said library of variant nucleic acid sequences encodes a polyclonal T cell receptor or T cell receptor

fragment having a naturally occurring diversity among the individual members of said polyclonal T cell receptor or T-cell receptor fragment.

35. The recombinant polyclonal manufacturing cell line according to any one of claims 32-34, wherein said collection of cells is derived from a mammalian cell line or cell type.

36. The recombinant polyclonal manufacturing cell line according to claim 35, wherein said mammalian cell line is selected from the group consisting of Chinese hamster ovary (CHO) cells, COS cells, BHK cells, YB2/0, NIH 3T3, myeloma cells, fibroblasts, HeLa, HEK 293, PER.C6, and derivative cell lines thereof.

37. A library of vectors for site-specific integration comprising a population of naturally occurring variant nucleic acid sequences, wherein each of said vectors comprises 1) one copy of a distinct nucleic acid sequence encoding a distinct member of a polyclonal protein that binds a particular antigen and 2) one or more recombinase recognition sequences.

38. The library according claim 37, wherein said population of naturally occurring variant nucleic acid sequences encode a polyclonal antibody or antibody fragment.

39. The library according claim 37, wherein said population of naturally occurring variant nucleic acid sequences encode a polyclonal T cell receptor T cell receptor fragment.

40. The library according to any one of claims 37-39, wherein each member of said library of vectors further comprises a recombinase encoding nucleic acid sequence.